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Validation of non invasive measurements of cardiac output in mice using echocardiography

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Abstract

Although multiple echocardiographic methods exist to calculate cardiac output (CO), they have not been validated in mice using a reference method. Echocardiographic and flow probe measurements of CO were obtained in mice before and after albumin infusion and inferior vena cava occlusions. Echocardiography was also performed before and after endotoxin injection. Cardiac output was calculated using LV volumes obtained from a M Mode or a 2D view, LV stroke volume calculated using the pulmonary flow, or estimated using pulmonary VTI. Close correlations were demonstrated between flow probe-measured CO and all echocardiographic measurements of CO. All echocardiographic-derived CO overestimated the flow-probe measured CO. 2D images-derived CO was associated with the smallest overestimation of CO. Interobserver variability was lowest for pulmonary VTI derived CO. In mice, CO calculated from 2D parasternal long axis images is most accurate when compared to flow probe measurements, however, pulmonary VTI-derived CO is subject to less variability.

Keywords

Mice; Doppler echocardiography; cardiac output; flow probe; sepsis

Introduction

Mice are used extensively in cardiovascular research, and evaluation of their cardiac output (CO) provides important information on their cardiac function. Cardiac output in mice can

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Disclosures

None

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be directly measured using a Doppler flow probe positioned on the ascending aorta^{1–4} or can be calculated from the left ventricular (LV) diastolic and systolic volumes measured by an intraventricular conductance catheter^{5–8}. Although these techniques have been extensively employed, they are invasive, time-consuming, and their performance disrupts the normal physiologic conditions. Serial follow-up of the CO using these techniques has not been attempted. Similarly, measurement of the cardiac output using transesophageal echocardiography has been described⁹; this technique however necessitates deep anesthesia and lengthy analysis. Magnetic resonance imaging also allows quantification of mice ventricular volumes¹⁰, and, therefore potentially of CO. Serial imaging of a large number of animals however using magnetic resonance imaging is difficult due to the length of acquisition, processing and expense.

Transthoracic echocardiography is increasingly used to assess mice cardiac phenotypes. Transthoracic echocardiograms are relatively easy to perform and allow non invasive serial measures of LV size and function^{11–13}. Multiple approaches have been proposed to calculate CO using echocardiography in humans^{14, 15}. Cardiac output may be similarly calculated in mice using transthoracic echocardiography. Feldman *et al.*⁶ reported that CO calculated using an M Mode method did not correlate to that measured with a flow probe; of note however, the approaches were not simultaneous as echocardiography was performed closed-chest. Other routinely used echocardiographic approaches to CO have not been validated and compared using a reference method in mice. Such validation is important as the mouse heart's size, shape and beating rate may invalidate techniques used in humans and large animals.

The objective of this study was to determine whether echocardiography is a reliable method to measure mouse CO, and what echocardiographic approach is most appropriate for this measurement. For this purpose, CO measured or estimated by a variety of echocardiographic methods (using the M Mode, 2D images and pulmonary flow) was compared to CO measured by an ascending aortic flow probe. The variability of each method was also tested.

Methods

Protocol

C57BL/6 male mice (25±2 g) were anesthetized with an intraperitoneal injection of ketamine (0.1 mg/g) and xylazine (0.01 mg/g) and placed supine on a heated operating table. After tracheotomy, mice were ventilated and a custom-made catheter (PE10, Becton Dickinson, Franklin Lakes, NJ) was inserted via the right carotid artery to monitor mean arterial pressure. A venous line was inserted in the left jugular vein. A thoracotomy was performed and a flow probe (1.5SL; Transonic Systems, Ithaca, NY) was placed around the ascending aorta. The probe was precalibrated in the manufacturer's factory under controlled procedures traceable to the National Institute of Standards and Technology¹⁶. This validation is done using a phantom with a roller pump and controlled liquid flows through customized tubing that does not interfere with the flow probe signal. The calibration is done every year by the manufacturer. Cardiac output was continuously displayed and recorded with a digital data acquisition system (DI720; Dataq Instruments, Akron, OH). In this well-controlled open-chest model, transthoracic echocardiography was performed in the decubitus position using a transducer centered on 30 MHz (RMV707B, Vevo 770, Visualsonics, Toronto, Ontario, Canada) with an axial resolution of 55 µm and a lateral resolution of 115 µm at a depth of 1.3 cm^{17, 18}. Both the flow probe and the echocardiography probe were held using mechanical arms. Because of frequency overlap between both ultrasound systems, Doppler echocardiographic and flow probe data could not be recorded simultaneously. The flow probe measurement was performed first and was immediately followed by the echocardiographic measurement. Data were measured during

steady-state conditions using an intravenous infusion of albumin (12.5% in normal saline, 50µl/min) and during CO decrease obtained by intermittent clamping of the inferior vena cava. The ventilator was turned off and measurements were obtained at end-expiration. The flow probe-measured CO was averaged over 5 consecutive cardiac cycles.

Echocardiographic measurements of CO

Three echocardiographic algorithms for CO measurement were compared to flow probe-measured CO (Figure 1). In methods 1 and 2, CO was calculated as the product of LV stroke volume (SV= LV end-diastolic volume- LV end-systolic volume) and heart rate. In method 3, CO was calculated as the product of pulmonary flow/cardiac cycle and heart rate. **Method 1. LV volumes derived from D^3 (M Mode).** Left ventricular end-diastolic and end-systolic volumes were calculated from a M Mode image of a parasternal short axis view at the level of the papillary muscles by the “ D^3 ” formula. Left ventricular end-diastolic (LVEDD) and end-systole (LVESD) diameters were measured and SV was derived from the difference of LV end-diastolic and end-systolic volumes calculated respectively as $LVEDD^3$ and $LVESD^3$ (Figure 1 Panel A). Cardiac output was calculated as the product of SV and heart rate. **Method 2. LV volumes derived from the area-length formula (parasternal long axis view).** The second method used a parasternal long axis 2 dimensional view. LV end-diastolic volume and LV end-systolic volume were calculated using a prolate-ellipsoid formula (Volume= $8.A^2/3\pi.L$, A= LV Area and L= LV length) as shown on Figure 1 (Panel B). SV was then calculated as the difference between these two volumes. **Method 3. Pulmonary flow.** The third method calculated the pulmonary flow as the product of the main pulmonary area at the level of the pulmonary valve tips and the velocity time integral of the pulsed wave Doppler of the pulmonary flow (VTI) at that level (Figure 1, Panel C). Both pulmonary measurements were obtained from a parasternal short-axis view at the aortic root level. The pulmonary flow VTI was also compared to flow probe results. All measurements were obtained by one observer blinded to the results of the flow probe and averaged on 5 consecutive cardiac cycles. The echocardiographic-derived M Mode measurements were obtained in 9 mice (36 measurements), the 2 dimensional measurements in 6 mice (33 measurements), the pulmonary measurements in 9 mice (34 measurements).

Ratio of the long to short axis of the LV in mice

In order to determine a possible cause of under or over-estimation of stroke volume and CO measurements by echocardiography, the ratio of the long axis to short axis was measured in the mice undergoing the 2D echocardiographic measures and in 5 additional closed chest control mice. These 5 control mice (C57BL6 2 months old male) were sedated using isoflurane 0.5–1% and a parasternal long axis view was obtained.

Application to endotoxin shock

A baseline echocardiogram was performed in ten C57BL6 2 month old male mice. Measurements of cardiac output were obtained using M Mode, 2 dimensional images, and pulmonary flow as described previously. Three days later, the mice were challenged with an intraperitoneal injection of *Escherichia coli* 0111:B4 endotoxin (LPS, 25 mg/kg). An echocardiogram was repeated 5 hours after the injection. In order to avoid hypothermia and dehydration, the mice were kept on a heated platform and received 0.5 ml of saline intraperitoneally 30 minutes prior to the echocardiogram.

Statistical analysis

Statistical analysis was performed using JMP 9.0 (SAS). Results are reported as mean \pm SEM. The prediction of flow probe measured CO in our experiments was first obtained by a Standard Least Squares model including the effect of the echocardiographic parameter of

interest, the effect of the mouse and the crossed effect of mouse*echocardiographic parameter. To compare the ability of each echocardiographic algorithm to predict flow probe-measured CO, the standard deviation of the residuals was calculated for each algorithm. The amplitude of the overestimation of the flow probe-measured CO by echocardiographic methods was compared using an ANOVA and Student's t-tests if appropriate. The comparison of the echocardiographic parameters before and after endotoxin was obtained using Student's paired t-tests. A value of $P < 0.05$ was considered significant. The intraobserver and interobserver variability of each echocardiographic method was expressed as mean \pm SD of the difference between 2 measurements. The variability between methods was compared using a test for Equality of Variances. If the test for Equality of Variances showed an overall difference between groups, post-hoc paired-wise comparison were performed.

Results

Hemodynamic measurements at baseline

At baseline with all catheters and flow probe in place, systolic arterial pressure was 70 ± 4 mmHg and heart rate 366 ± 19 bpm in the mice used for M Mode measurements, 72 ± 6 mmHg and 445 ± 24 bpm in the mice used for 2D measurements and 72 ± 7 mmHg and 373 ± 22 bpm in the mice used for pulmonary measurements

Relationship between echocardiographic- and flow probe-measured CO

Cardiac output measured by flow probe correlated closely to CO calculated using M Mode (Flow probe-measured CO = $(0.38 \text{ M Mode-calculated CO}) + 0.78$, $r^2 = 0.78$, $p < 0.0001$, Figure 2A upper Panel), 2D images (Flow probe-measured CO = $(0.64 \text{ 2D-calculated CO}) + 0.9$, $r^2 = 0.87$, $p < 0.0001$, Figure 2B upper Panel), and pulmonary flow (Flow probe-measured CO = $(0.48 \text{ pulmonary flow-calculated CO}) + 0.84$, $r^2 = 0.78$, $p < 0.0001$, Figure 2C upper Panel). There was also a close correlation between CO measured by flow probe and estimated by the pulmonary velocity time interval (VTI) (Flow probe-measured CO = $(2.34 \text{ VTI-derived CO}) - 0.13$, $r^2 = 0.93$). The standard deviations of the residuals were 1 ml/min (VTI), 1.4 ml/min (pulmonary flow and 2D) and 1.5 ml/min (M Mode).

All echocardiographic methods (M Mode, 2D images, pulmonary flow) overestimated the flow probe measured CO. Before vena cava occlusion, flow probe-measured CO was 10 ± 1 ml/min in the mice used for M Mode measurements, 11 ± 1 ml/min in the mice used for 2D measurements, 10 ± 1 ml/min in the mice used for pulmonary measurements. Cardiac output measured near-simultaneously using echocardiography was 21 ± 1.3 ml/min (M Mode), 14 ± 1 ml/min (2D images), 17 ± 0.8 ml/min (pulmonary flow). When the measures before and during caval occlusion were analyzed, the overestimation of the flow probe-measured CO remained greatest for the M Mode (1.3 ± 0.8 fold, $p < 0.05$ compared to the 2 other methods, Figure 2A lower Panel). Two-dimensional images-calculated CO overestimated flow probe-measured CO to a lesser extent than the other echocardiographic methods (overestimation of 0.3 ± 0.5 fold, $p < 0.05$ compared to the 2 other methods, Figure 2B, lower Panel). Cardiac output estimated from the pulmonary flow overestimated flow probe-measured CO by 0.8 ± 0.5 fold (Figure 2C, lower panel).

Intra- and inter-observer variabilities

Intra- and inter-observer variabilities of the echocardiographic measurements are reported in Table 1. The intraobserver variabilities were not statistically different between methods. Of note, the intra- and inter- observer variabilities of the pulmonary artery diameter (expressed as the standard deviation of the % error) were 7%. The inter-observer variability of the CO derived from the pulmonary VTI was lower than that of all other methods.

Ratio of the long to short axis of the LV in mice

The ratio of the LV long axis to short axis was 2.1 ± 0.1 at end-diastole and 3.6 ± 0.4 at end-systole in the open-chest mice and 1.8 ± 0.1 at end-diastole and 2.9 ± 0.1 at end-systole in closed-chest mice.

Application to endotoxin-induced shock

The COs calculated using M Mode, 2D images and pulmonary flow before and after endotoxin injection are reported in Table 2. Before endotoxin injection, the 2D images method yielded lower values of CO than the pulmonary flow or the M Mode methods. After endotoxin injection (lower COs), both the 2D images and the pulmonary flow method yielded lower values of CO than the M Mode.

The decrease in CO induced by endotoxin was detected by all methods. However, the decrease detected by pulmonary flow and pulmonary VTI ($50 \pm 6\%$ and $59 \pm 4\%$ respectively) was more profound than the decrease detected by M Mode and 2D images ($26 \pm 6\%$ and $36 \pm 8\%$, both $P < 0.01$ vs. the decrease detected by pulmonary flow and VTI). The variability of the decrease was lower when calculated using VTI than with the other methods.

Discussion

The present study demonstrates that CO can be accurately estimated using echocardiography. Four echocardiographic methods were tested including M Mode-, 2D-, and pulmonary flow-calculated CO, and pulmonary VTI-derived CO. All echocardiographic methods correlated closely with flow probe-measured CO. Echocardiographic measurements overestimated CO measured by flow probe. The overestimation was greatest using the M Mode and lowest using 2D images. Intraobserver variability was similar using all algorithms however, interobserver variability was lowest using pulmonary VTI. In a model of mouse endotoxin-induced shock, the values of CO reported using M Mode were the highest. Values of CO reported using 2D images were the lowest. Pulmonary flow and pulmonary VTI detected a greater decrease in CO after endotoxic injection than 2D or M Mode.

Cardiac output is routinely measured using echocardiography in humans and large animals. Recently, CO was validated in rats by comparing echocardiographic measurements to thermodilution¹⁹. Measurement of the CO by diameter and VTI measurements at the level of the aortic annulus were found to have the greatest accuracy and the smallest bias. In mice, the absence of reproducible apical views precludes the accurate measurement of flow velocities at the level of LV outflow tract. The LV outflow tract velocities may be measured on the parasternal long axis view, however the orientation of the aortic root on that view is not parallel or close to parallel to the Doppler beam. In contrast, the pulmonary artery is easily visualized using a parasternal short axis view, the orientation of the pulmonary artery is parallel to the Doppler beam, and the pulmonary valve is seen, allowing to place the Doppler cursor reproducibly at the same location in all mice (tip of the leaflets). Similarly, the M Mode and parasternal long axis views of the LV may be easily obtained in all mice.

Cardiac output was measured using a flow probe positioned around the ascending aorta. This method has been used in mice by our team¹ and others^{3, 4, 6}. Janssen *et al.* found that CO measured using a flow probe in awake mice was of 16 ml/min³, a value greater than that found in our study. However, in a subsequent study, the same investigators reported a significant decrease in flow probe-measured CO using anesthesia, with values similar to our results⁴. Likewise, using a conductance catheter, Pacher *et al.*⁷ reported values of CO between 6 and 10 ml/min in anesthetized mice.

All echocardiographic methods overestimated the flow probe-derived CO. Although the flow probe did not take into account the coronary artery flow, the underestimation of the CO linked to coronary flow represents approximately 10% of the CO²⁰ and cannot be the only explanation to the differences observed in our study.

As reported previously in rats¹⁹, the greatest overestimation of the flow probe-derived CO was noted with M Mode measurements. Similarly, the highest values of CO in mice before and after endotoxin injection were reported using M Mode. Calculation of LV volume using the M Mode and equating LV volume to the cube of its short axis assumes that the long axis of the LV has twice the length of the anteroposterior diameter. As shown by our results, this ratio appears to be valid in mice in diastole however it is much greater in systole, as the long axis of the LV does not decrease as much as the short axis. The LV end-systolic volume is therefore underestimated using the M Mode calculation and the CO overestimated. Another factor that may play a role in the overestimation observed using the M Mode is the fact that the LV may not be perfectly perpendicular to the ultrasonic beam, leading to an overestimation of both end-systolic and end-diastolic diameters. As the CO calculation is based on the cube of each diameter, the impact of this error will affect the larger volume (end-diastole) to a greater extent than the smaller volume (end-systole), inducing an overestimation of the stroke volume and of the CO.

The overestimation of the CO by the pulmonary flow measurement has also been reported in rats¹⁹. The major source of error in the measurement of pulmonary flow is the pulmonary artery diameter measurement, due to poor visualization of the walls of the main pulmonary artery²¹. The difficulty in the estimation of the pulmonary artery diameter is underscored in the present study by the greater inter-observer variability found in the calculation of pulmonary flow than in pulmonary VTI. However various loading changes did not significantly impact the measured diameter of the pulmonary artery since the greatest observed standard deviation for a unique observer was 10 μ m, far below our spatial resolution. Pulmonary VTI was also able to detect a decrease in the mouse CO after endotoxin injection that was more pronounced and less variable than the other methods of CO measurements.

The degree of overestimation of the CO was less using the 2 dimensional images than that of the M Mode or pulmonary flow. Similarly, in mice after endotoxin injection, CO measurements using 2 dimensional images were lower than measurements using M Mode or pulmonary flow. Investigators had previously noted that algorithms based on 2D images were more accurate than those based on M Mode for measurement of LV mass in mice²². Two-dimensional echocardiography still overestimated the flow probe-measured CO, suggesting either an overestimation of the LV end-diastolic volume or an underestimation of LV end-systolic volume. Pacher *et al.*⁷ reported end systolic volumes in mice between 9 and 20 μ l using a conductance catheter; using echocardiography our volumes were smaller (from 2 to 11.5 μ l). It is conceivable that analysis of the echocardiogram underestimates LV systolic volume as a very small LV cavity may not be totally accurately traced. Furthermore, on the parasternal long axis view of the mouse, it is difficult to avoid and exclude the papillary muscle from the area measured, in particular in systole. Not avoiding the papillary muscle would also underestimate LV end-systolic volume and participate in an overestimation of CO.

Limitations. Due to the presence of the flow probe in the suprasternal area, we were not able to reproducibly obtain aortic flow parallel to the Doppler beam. This limitation precluded us from comparing the methods tested to the measurement of CO using aortic flow¹⁹. For the 2D LV volume assessment, although the definition of the papillary muscles insertion may be seen better in a short axis plane, we used the parasternal long axis view. The parasternal

long axis view allowed us to use area and LV length measured within the same cardiac cycle. Finally, due to the technical complexity of our model, we had to use a well-controlled open-chest model and variability of CO measurement could be expected to be higher in conscious mice.

In conclusion, echocardiography can noninvasively measure CO in the mouse, with close correlations to flow probe measures. Although overestimation of the flow probe-measured CO is noted whatever the echocardiographic method used, the close correlations noted between each echocardiographic method and flow probe-measured CO allow an acceptable estimation of CO and, more importantly, an accurate assessment of its changes within and between mice. Investigators may either choose to report CO calculated from the pulmonary VTI, a method with low variability or CO derived from 2D images, the approach that gives the least overestimation of the flow probe-measured CO.

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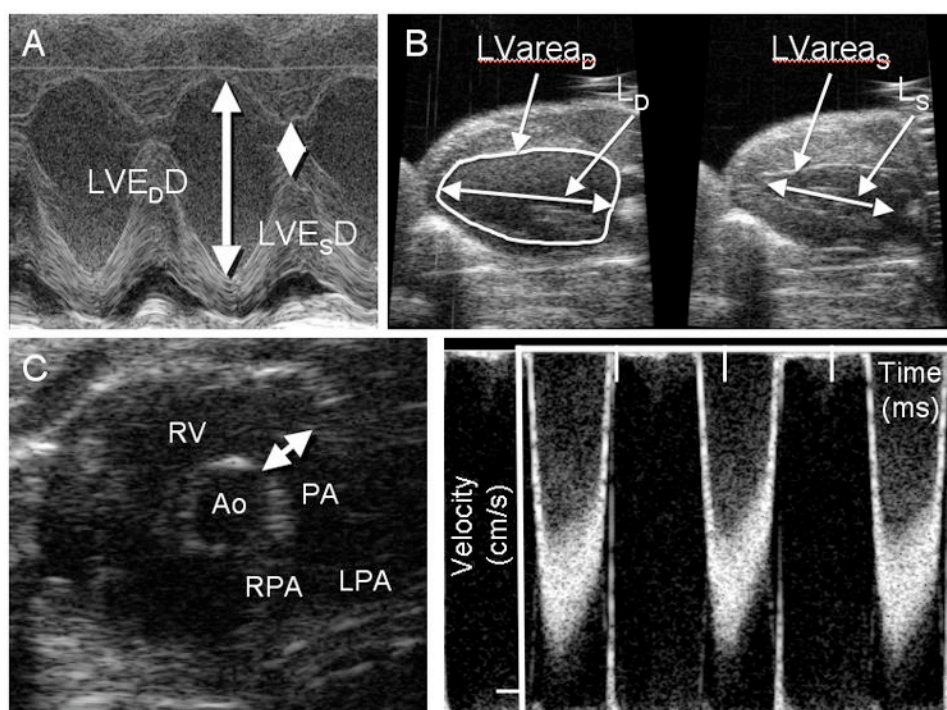


Figure 1. Echocardiographic acquisitions used to calculate CO. Panel A. M Mode ($CO = (LVE_D^3 - LVE_S^3) \times \text{heart rate}$), Panel B. 2D ($CO = (8LVarea_D/3\pi L_D - 8LVarea_S/3\pi L_S) \times \text{heart rate}$), Panel C. Pulmonary flow. On the left, parasternal short axis view at the level of the aortic root showing the pulmonary artery diameter (arrow). On the right, pulmonary velocity time integral ($CO = \pi \cdot \text{pulmonary diameter}^2/4 \times \text{pulmonary VTI}$). Each bar on the x axis corresponds to 100 ms, each bar on the y axis corresponds to 1m/s. LVE_D: left ventricular diameter at end-diastole, LVE_S: left ventricular diameter at end-systole, LVarea_D: left ventricular area in the parasternal long axis view at end-diastole, LVarea_S: left ventricular area in the parasternal long axis view at end-systole, L_D: left ventricular long axis at end-diastole, L_S: left ventricular long axis at end-systole, Ao: aortic valve, RV: right ventricle, PA: main pulmonary artery, RPA: right pulmonary artery, LPA: left pulmonary artery.

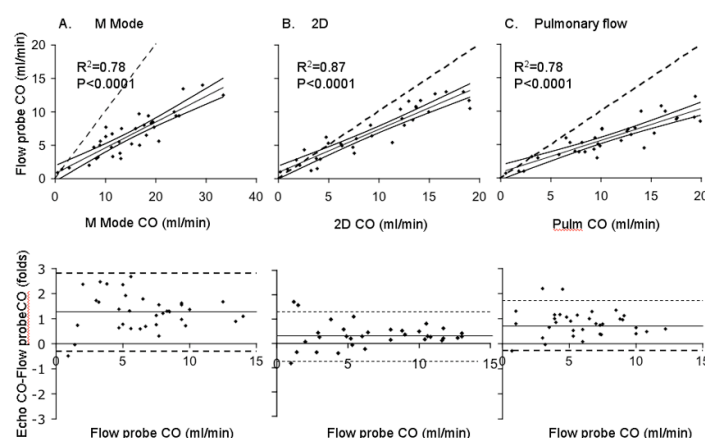


Figure 2.

Relationship between CO calculated from echocardiographic algorithms and CO measured using a flow probe. Upper Panels: Linear regression analyses comparing the CO calculated by echocardiography and measured by flow probe. The 25 % and 75% confidence interval of the estimate are also shown. The dashed line represents the line of identity. Lower Panels: Agreement between the CO calculated by echocardiography and measured by flow probe. Horizontal reference lines are zero fold difference (bold line), bias of the echocardiographic method (fine line) and 2SD above and below the bias (dashed lines). Panel A. Cardiac output derived from the M Mode images, Panel B. Cardiac output derived from the parasternal long axis 2D view, Panel C. Cardiac output estimated from the pulmonary flow. The echocardiographic-derived M Mode measurements were obtained in 9 mice (36 measurements), the 2 dimensional measurements in 6 mice (33 measurements), the pulmonary measurements in 9 mice (34 measurements).

Table 1

Intra- and inter-observer variabilities of the echocardiographic algorithms measuring CO.

<i>Algorithm used to measure CO</i>	Intra-observer variability of CO measurement	Inter-observer variability of CO measurement
M Mode	-0.5 ± 1.22 (-1.9 ± 6.6)	0.2 ± 1.8 (0.7 ± 10.2)
2D	-0.1 ± 1.0 (2.3 ± 9.4)	-0.5 ± 1.5 (-8.9 ± 14.5)
Pulmonary Flow	0.2 ± 1.1 (1.5 ± 8.4)	-0.9 ± 1.5 (-6.1 ± 10.2)
VTI	0.0 ± 0.14 (-0.7 ± 4.7)	0.0 ± 0.17 (-0.9 ± 6.6)

Intra- and inter-observer variabilities are calculated as the average \pm SD of the error in repeated measurements of CO. Results are expressed in ml/min and in % error (in parentheses).

Table 2

Echocardiographic parameters before and after endotoxin injection.

	HR	LVID	FS	CO-MM	CO-2D	CO-pulm	pulmVTI
Baseline	445±19	3.8±0.1	38±2	18±1	12±1 [‡]	18±2	3.7±0.2
5 hours after LPS	512±11	3.9±0.1	17±1.2	13±1	7±1 [*]	8±1 [*]	1.5±0.1
P Value	0.02	0.47	<0.0002	0.01	0.03	0.0003	<0.0001

Values are mean ± SEM. N=10 mice. HR; heart rate (beats per minute), LVID; left ventricular internal diameter in diastole (mm), FS; fractional shortening (%), CO-MM (ml/min); cardiac output calculated using M Mode (ml/min), CO-2D; cardiac output calculated using 2 dimensional images (ml/min), CO-pulm; cardiac output calculated using the pulmonary flow (ml/min), pulm VTI; pulmonary velocity time integral (cm), LPS; *Escherichia coli* 0111:B4 endotoxin. P value; p value of the comparison between baseline and 5 hours after LPS,

^{*} P<0.001 vs. CO-MM at the same time point,

[‡] P<0.001 vs. CO-pulm at the same time point.